B3

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 7/40, 9/06, 31/23, 35/36

A1

(11) International Publication Number: WO 98/33474

(43) International Publication Date: 6 August 1998 (06.08.98)

(21) International Application Number: PCT/US98/01587

(22) International Filing Date: 30 January 1998 (30.01.98)

(30) Priority Data:
60/036,767 31 January 1997 (31.01.97) US

(71)(72) Applicants and Inventors: HOYT, Kenneth [US/US]; 12300 Buggy Lane, Jones, OK 73049–8702 (US). LEMLEY, Paul [US/US]; 759 Hoffman Road, Gettysburg, PA 17325 (US).

(74) Agent: HENDRICKS, Glenna; P.O. Box 2509, Fairfax, VA 22031–2509 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: USE OF OIL FROM EMU OR RHEA BIRDS AS TRANS-MEMBRANE CARRIERS FOR DELIVERY OF DRUGS, PEPTIDES AND VACCINES

(57) Abstract

This invention provides a method for transdermal transportation of proteins, peptides and other therapeutic agents using oil obtained from the sebaceous glands of rhea and emu birds. The method may also be used to facilitate transport across other animal membranes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Paso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Title: USE OF OIL FROM EMU OR RHEA BIRDS AS TRANS-MEMBRANE CARRIERS FOR DELIVERY OF DRUGS, PEPTIDES AND VACCINES.

Field of the Invention:

This invention relates to use of oil from birds, particularly from the emu and rhea birds. Compositions containing the oil of the birds as carriers for therapeutic or immunogenic agents are disclosed.

Background of the Invention:

Use of oil from the emu bird for purposes of obtaining medicinal benefit has been known. U.S. Patent 5,472,713 discloses use of emu oil for lowering cholesterol, triglycerides and low density lipoproteins, for increasing high density lipoproteins and for other medicinal purposes. The oil is administered parenterally, orally, rectally or topically to the mucosa. In the later instance, application is to the mucosa. The emu oil does not act as a carrier for other active agents.

U.S. Patent 5,431,924 teaches use of emu oil as an anti-inflammatory agent. That patent teaches and claims a method of preparing a yellow extract of emu oil as an anti-inflammatory and relies on other agents to transport of the oil across the dermis or mucous membranes. The emu oil extracts for anti-inflammatory purposes are prepared using organic solvents. As taught therein, it is necessary to prepare compositions for anti-inflammatory use by adding to the yellow emu oil obtained by the methods of '924 certain diluents to potentiate the anti-inflammatory activity of the emu oil and to act as transport agents. There is no suggestion therein that emu oil, in and of itself, acts as a transport agent for other active agents such as pharmaceuticals and vaccines.

Summary of the Invention:

This invention provides a method for transdermal transportation of proteins, peptides and other therapeutic agents. The method may also be used to facilitate transport across other animal membranes.

A preferred method of preparing the oil for use in accord with the objectives of the invention comprises the steps of 1)

breaking up the tallow from the sebaceous gland; 2) rendering the tallow prepared in step 1 in the presence of steam at a temperature of about 140°F; 3) drawing off the oil rendered in step 2; 4) straining the product of step 3 to remove foreign and extraneous protein; and 5) removing the remaining water from the oil.

Compositions of the invention are prepared by adding active agents such as peptides, proteins or other active therapeutic or disease-preventing agents to the emu oil. These compositions are then applied to the skin or other epithelial tissue, including the mucosa.

Detailed description of the invention:

It is the purposes of this invention to provide improved means for transdermal administration of active agents, including medicinals and vaccines, across the dermal barrier using oil obtained from the sebaceous glands of flightless rhea and emu birds. The method described herein is particularly useful for transport of peptides and proteins. By means of the invention it is possible to administer vaccines without use of sterile equipment or solutions. This benefit will be especially appreciated by those responsible for administering antigens in countries where refrigeration and sterile materials and equipment are not readily available. The methods of the invention make it possible to vaccinate populations without needles and to administer prophylactic or therapeutic proteins, peptides and other active agents without breaking the skin. The use of the oil also facilitates transport across cell membranes.

The methods of the invention are of special value for use in administration of lipophilic compounds such as steroids which may be detoxified in the liver. For example, testosterone can be less toxic when administered in a manner that bypasses the liver. Other active agents such as analgesics, antihistamines, anesthetics, lipophilic antimicrobials, anti-inflammatory agents, anti-cancer agents, oil-soluble vitamins, etc. may be administered by the methods of the invention.

Materials and Methods:

Preparation of the oil:

A preparation of oil from the rhea bird was made. Adipose tissue from the sebaceous gland from the bird was ground in a meat grinder to break down the tallow and allow larger surface areas. The ground tallow was then rendered in a steam kettle at a temperature of 140°F. The oil was drawn off as it was rendered from the adipose tissue.

When most of the oil had been rendered from the tissue, the oil was strained in a 10 micron filter using a vacuum pump. (The oil was maintained at 140°F during this process.) After the oil had been strained and all foreign and extraneous protein removed, the oil was again heated to 140°F. The oil was maintained at that temperature until all water had evaporated from the oil. (Approximately six hours was required.) The oil so obtained was strained again by the method described above. The oil was then placed in glass containers and vacuum sealed using a pressure cooker at 15 pounds pressure for 20 minutes.

Using the procedure described above results in oil containing all of the desired constituents while avoiding oxidation that might occur when the oil is processed at higher temperatures. No other materials such as alcohol, carotenoids or other foreign components were added. Emu oil may be prepared in the same manner for purposes of this invention.

It is known that rhea and emu oils contain a number of different fatty acid chains. U.S. Patent 5,431,924 to Ghosh, et al. teaches emu oil made by fractionating the oil with hexane using a florisil column, eluting the column with hexane, dichloromethane and methanol and separating a biologically active yellow colored component from the oil to obtain a yellow residue. It is obvious that the method of preparing emu oil as taught herein is far simpler than that of Ghosh, et al.

Use of oil as a carrier:

In order to test the invention, antigens were mixed with the oil obtained by the methods described above. The oil was then applied to a clean-shaven patch of skin of mice. During testing, 30 to 50 μl of oil was used for each administration.

However, amounts of oil used for administration in accord with the teaching of the invention may vary depending on the agent to be administered. It is expected that the amount used will usually be in amount of 1 to 1000 μl per dose. The method was tested using agents of varying molecular weights (<2000 daltons to >60,000 daltons) and using both naturally-occurring and recombinant proteins.

Because it is easy to test response to antigens, antigenic proteins were administered and the level of antibody production evaluated. The test animals were pre-bled and tested to assure that the animal had no antibodies against the immunogen to be administered in the compositions.

For controls, the antigenic materials were mixed with water and rubbed on the control mice.

# mice	protein	Wt. (daltons)	Oil	# applications
6	6.0 μg conotoxin G1	1760	30 μl	1
6	3.0 μg "		50 μl	2
6	3.0 μg Frag. C *	30,000	30 μl	1
6	3.0 μg Frag. C *	30,000	30 μl	2
10	5.0 μg Staph. En. B**	28,000	30 μl	2
12	5.0 μg Ricin A	31,000	50 μl	2
6	3.0 μg Ricin A-B	60,000	30 μl	2

* Recombinant botulinum neurotoxin A, "c" fragment.
** Staphylococcal enterotoxin B

In each instance, 6 control animals were treated with the same proteins administered to the skin in water. In no instance did the controls show production of antibodies raised in response to the immunogen administered.

All animals treated in the manner described showed antibody titer of at least 10^2 to the antigen administered when the antigen was administered in the bird oil. (See table above.)

The administration of other active agents such as steroids and other anti-inflammatory agents, antihistamines, therapeutic and protective peptides using methods of the invention will

provide means of avoiding the breaking of the skin during administration.

The following exemplify the invention and are not meant to operate as limitations on the invention.

An anti-allergic composition is prepared using 1 ml of oil prepared by the method described above having added thereto 5 mg dexamethasone. The resulting composition is administered by application to the skin.

A nine amino acid sequence known as delta sleep inducing peptide (DSIP) of the structure Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu is formulated by adding D50 μl DSIP to 100 μl emu oil prepared in accord with the teachings above.

Many other peptides could be formulated in a similar manner. Such peptides include splenopentin (SP-5) having the structure Arg-Lys-Glu-Val-Tyr. This peptide is effective for inducing T-cell differentiation and for modulation of neuromuscular transmission. (Proc. Natl. Acad. Sci. USA 81: 2847-2847 (1984)) Others include vasoactive intestinal peptide (VIP) or biotinyl-VIP from human, porcin, chick, rat, or others, having the sequence His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 for prevention of cell death caused by human immunodeficiency virus (Nature 335: 639-642 (1984)) and for pharmacological treatment of tissues involving neuromuscular transmission (Arch. int. Pharmacodyn 305, 14-24 (1990)).

What we claim is:

1. A method of preparing oil from the emu or rhea bird comprising the steps of:

1) breaking up the tallow from the sebaceous gland;

2) rendering the tallow prepared in step 1 in the presence of steam at a temperature of about 140°F;

3) drawing off the oil rendered in step 2);

4) straining the product of step 3 to remove foreign and extraneous protein; and

5) removing the remaining water from the oil.

2. A composition of matter comprising a physiologically active agent containing as the primary carrier oil from an emu or rhea bird.

3. A composition of claim 2 wherein the oil is from a rhea bird.

4. A composition of claim 2 wherein the physiologically active agent is a protein.

5. A composition of claim 2 wherein the physiologically active agent is a recombinant protein.

6. A composition of claim 2 wherein the physiologically active agent is an immunogen.

7. A composition of claim 6 wherein the immunogen is a peptide.

8. A composition of claim 2 wherein the active agent is a peptide.

9. A composition of claim 2 wherein the active agent is a steroid.

10. A composition of claim 2 wherein the active agent is a neuropeptide.

11. A method of formulating a active agent chosen from a pharmaceutical or an immunogen comprising adding said pharmaceutical or immunogen to oil from an emu or rhea bird.

12. A method of claim 11 wherein said oil has been prepared in such a manner that oxidation of said oil has been essentially avoided.

13. A method of claim 11 wherein the active agent is a peptide.

14. A method of claim 13 wherein the peptide is an immunogen.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01587

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 7/40, 9/06, 31/23, 35/36
US CL :424/522, 434, 435, 436
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/522, 434, 435, 436

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,472,713 A (FEIN, et al) 05 December 1995, see col. 3.	1 --- 12
X --- Y	WO 96/34596 A2 (HOLICK, et al) 07 November 1996, see entire document.	2-4,9,11 --- 5-8,10,13,1 4
Y,P	US 5,725,858 A (FIORETTI, et al) 10 March 1998, see col. 1, lines 55-65.	2,3
X	WO 92/08470 A1 (EMU PRODUCTS WESTERN AUSTRALIA PTY. LTD.) 29 May 1992, see page 7, lines 11-24.	2,3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
16 MARCH 1998

Date of mailing of the international search report
07 MAY 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MARY TUNG
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01587

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, JPO, EPO, PATOSWO, MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS
Emu, rhea, tallow, oil, sebaceous, therapy, treatment, pharmaceutical